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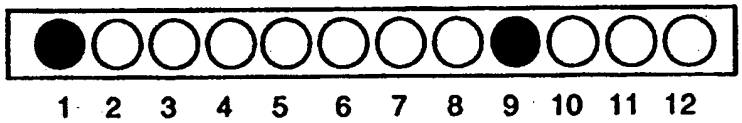
(54) Title: IDENTIFICATION OF ALLELES OF TANDEM REPEATS BY HYBRIDIZATION

(57) Abstract



The hypervariable region of human DNA due to the tandemly repeated DNA is useful in forensic identity test and human genetics. The alleles of such DNA have been identified by sizing by the gel electrophoresis either after restriction digestion or after amplification by the polymerase chain reaction. We have developed an easy and faithful alternative for identification of alleles of the tandemly repeated DNA based on the allele-specific hybridization and ELISA-technology. An aliquot of PCR-amplified digoxygenin-labeled sample DNA is mixed with a set of biotin-labeled standard DNA and the mixture is denatured and then renatured. Only those duplex DNA made of one strand from the sample and the other strand from the standard can be immobilized to the avidin-coated solid support and be attached by the antibody-conjugated enzyme producing visible precipitate. From the position of color development, the alleles in the sample are determined.

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Identification of alleles of tandem repeats by hybridization

Technical Field

Forensic medicine and human genetics

Background Art

Human DNA contains hypervariable sites caused by the varying number of repeated sequences (Nakamura et al., 1987; Weber and May, 1987). Each variety is an allele which can be recognized by different sizes of fragments. A few such loci used together can distinguish any two indivisuals who are not twins by generating an indivisual-specific allele combinations (Gill et al., 1985; Wong et al., 1987). The DNA profile obtained from such allele combinations revolutionized the art of human identification in forensic medicine (Kirby, 1990). The variable sequences are also useful in tagging a disease gene. The target gene, or more precisely a disease-causing allele, could be followed indirectly by the allele of the nearby hypervariable site (Antonarakis, 1989). It thus greatly facilitated pre-natal diagnosis and carrier detection. Because they provided markers so frequent and so easily detected, human genome mapping would not have been possible without them (Watson, 1990; Weissenbach et al., 1992).

The variability is usually manifested by the variations of lengths of DNA segment produced either by the digestion with a restriction enzyme (RFLP; restriction fragment length polymorphism) or by the amplification of specific segment of the DNA by PCR (polymerase chain reaction) (AFLP or AMP-FLP; amplified fragment length polymorphism) and subsequent gel electrophoresis. The AFLP is the method of choice nowadays surpassing the classical Southern-hybridization-based RFLP method because of its easy protocols, high sensitivity and frequent occurrences. However, the allele-identification by AFLP is still dependent on gel electrophoresis, which is responsible for the major part of the effort and time. In this proposal, we are presenting a new method of allele-identification based on hybridization.

Although alleles of a hypervariable site share a large part of the sequences due to the repeated core sequence, different alleles do not usually form hybrid. We investigated this phenomenon extensively (unpublished results). In Figure 1, the outcome of a

allele-mixing experiment is schematically shown. In lane 1 and lane 2, two alleles are shown as amplified by PCR. In lane 3, the two alleles are mixed, denatured and then renatured. It shows that the two alleles segregate inspite of their extensive homology of sequence. The segregation of alleles during hybridization suggests that the alleles could be identified by hybridization to a set of standard alleles. In fact, such identification of alleles by hybridization was not unprecidented.

Human leucocyte antigen (HLA) provided another class of hypervariable genetic locus which is also widely utilized for human identification. The variability is not because of the different number of repeated sequences but because of the small differences in sequence. The allele in a sample is determined by hybridization to a series of membrane-fixed allele-specific oligonucleotides (Saiki et al., 1989). The unbound sample DNA is washed away and the hybridized DNA is located by the sample DNA-specific color development.

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Disclosure of Invention

We discovered that an allele of a tandemly repeated sequence such as VNTRs (variable number of tandem repeats) or STRs (short tandem repeats) do not hybridize to other alleles with different number of repeats under appropriate conditions. The major obligatory condition is that the alleles in hybridization must be flanked with unique sequences as in PCR (Figure 1). The allele-specific hybridization could be easily detected by standard ELISA technology (For general immunological as well as molecular biological techniques, consult Ansubel et al., 1989). It will obviate the gel electrophoresis step, which is tedius and labor-intensive, in the traditional allele-identification of such hypervariable loci. Thus, the new method will greatly speed up the overall procedure and save efforts. Unlike in HLA, the number of applicable sites are numberous and most of the alleles can be discriminated.

For any given hypervariable locus, a set of standard DNA representing major alleles is to be prepared. In Figure 2, the overall procedure of allele-specific hybridization is shown in sequence. The standard DNA is tagged at the 3'-end, for example, with biotin-dUTP (black knob) using the terminal deoxynucleotidyl transferase. The sample DNA is amplified by PCR in the presence of any modified nucleotide, for example, digoxygenin-conjugated dUTP (circle), which provides the anchor for the enzyme as described below. For simplicity, only one strand is depicted. In the diagram, two hypothetical alleles, 1 and 9, are mixed with the standard allele 1. The DNA is then applied to a microtiter plate coated with avidin (large oval). Only allele 1 of the sample hybridizes to the standard allele 1 and the allele 9 DNA is washed away in the next stage. Therefore, although all standard DNA will be immobilized on the microtiter plate, only standard allele 1 and 9 can retain digoxygenin-labeled sample DNA. The anti-digoxygenin-antibody-conjugated horseradish peroxidase (Anti-Dig HRP) is added and attached to the sample allele. Unbound enzymes are washed away and the

production of colored precipitate by the enzyme mark the location of the bound sample DNA, which is in turn the identity of the sample allele. In Figure 3, the color developed in the slots of allele 1 and 9 out of 12 hypothetical standard alleles suggesting that the imaginary sample had two alleles, 1 and 9.

Brief Description of Drawings

Figure 1: A diagram depicting allele-segregation on an agarose gel. In lane 3, the two alleles, of lane 1 and lane 2, are mixed, denatured and renatured. It shows that the two alleles of a tandemly repeated sequence segregate during the hybridization.

Figure 2: Procedure of the hybridization and subsequent detection on a solid support. The standard DNA is tagged with biotin (black knob) and the sample DNA is labeled with digoxygenin (circle). The standard and sample DNA is mixed, denatured, renatured and then applied to a avidin (large oval)-coated microtiter plate. The standard DNA and its hybridized sample DNA are immobilized to the well due to the biotin-avidin interaction. The horseradish peroxidase (gray square) anchored on the sample DNA via conjugated anti-digoxygenin antibody catalyzes the conversion of colorless substrate to colored precipitate.

Figure 3: Allele-identification by hybridization to a set of standard DNA. On a hypothetical 12-allele set of a hypervariable locus, allele 1 and 9 of a sample are detected by the developed color precipitate.

Best Mode for Carrying Out the Invention

For each hypervariable locus, a kit consisting of the standard DNA representing major alleles of the locus is prepared. The standard DNA is conjugated with biotin as described above. They are provided within a series of tubes representing the alleles.

The kit may include all the necessary components for PCR like primers and reaction buffers as well as those for the hybridization and color development for optimal detection.

Industrial Applicability

The kits can be made for all known DNA polymorphic sites due to tandem repeats. A few kits in combination will provide an efficient diagnostic tool for human

identification in forensic and paternity test. The tracing of alleles for pre-natal diagnosis or carrier detection by the gel electrophoresis can be subsitituted with kits for the corresponding loci for better efficiency. Even new genes responsible for a disease can be pursued and mapped with a series of kits representing polymorphic sites nearby the disease gene.

Claims

1. Identification of PCR-amplified alleles of tandemly repeated DNA by hybridization to a set of standard alleles.

Figure 1

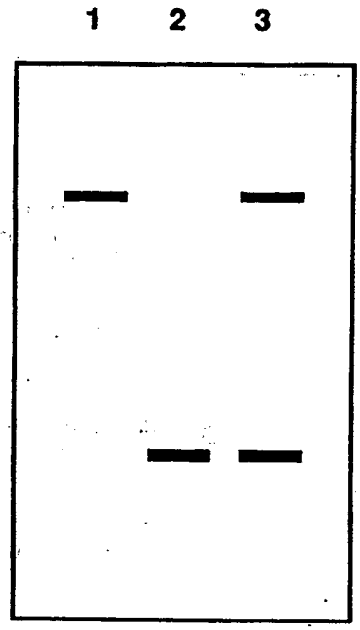
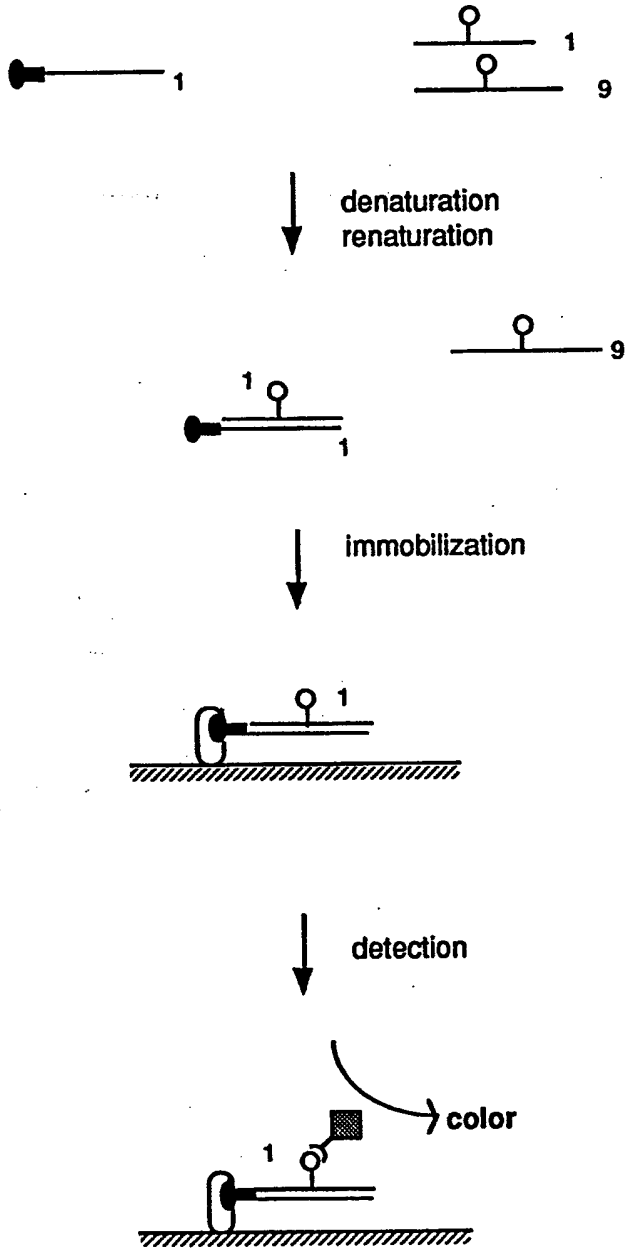


Figure 2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1
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denaturation
renaturation
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detection
color
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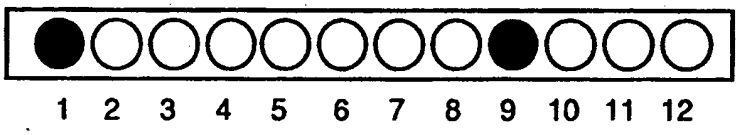
Figure 3



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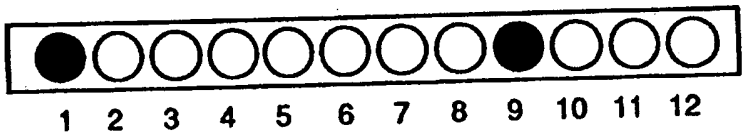

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 94/00117

A. CLASSIFICATION OF SUBJECT MATTER

IPC[6]: C 12 Q 1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC[6]: C 12 Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS, WPIL: tandemly w repeated w dna, PCR, identif+

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Chemical Abstracts, vol. 118, no. 7, issued 1993, February 15 (Columbus, Ohio, USA), P.E.WARBURTON et al. "PCR amplification of tandemly repeated DNA: analysis of intra- and interchromosomal sequence variation and homologous unequal crossing-over in human alpha satellite DNA." page 182, abstract no. 53228s & Nucleic Acids Research 1992, 20(22), 6033-42.	1
P,A	Chemical Abstracts, vol. 120, no. 9, issued 1994, February 28 (Columbus, Ohio, USA) C.C.LIN et al. "Isolation and identification of a novel tandemly repeated DNA sequence in the centromeric region of human chromosome 8." page 337, abstract no. 97927q & Chromosoma 1993, 102(5), 333-9.	1

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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